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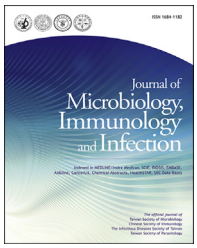

ORIGINAL ARTICLE

Antimicrobial susceptibility of clinical *Enterobacteriaceae* isolates at the emergency department in a regional hospital: A threat of extended spectrum beta-lactamase-producers among nursing home residents

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Abstract *Background/Purpose:* The prevalence of extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* in nursing home residents has rarely been reported in Taiwan.
Methods: A retrospective study was performed at medical wards of a district hospital at southern Taiwan between July 2009 and June 2011. Patients were included if they were older than 18 years, admitted via the emergency department, and their blood, sputum, or urine culture revealed the growth of *Escherichia coli*, *Klebsiella pneumoniae*, or *Proteus mirabilis*. From each patient only the first isolate from the infection site was included. Antimicrobial susceptibility was determined using the disc diffusion method.

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Results: Overall, 827 patients were included, with 354 (42.8%) coming from the community and 473 (57.2%) referred from a nursing home. Of the isolates acquired in nursing home, 45.5% (215/473) harbored ESBL. By contrast, 20.6% (73) of 354 isolates acquired in the community exhibited the ESBL production phenotype ($p < 0.001$). Of the isolates obtained from blood, urine, or sputum, 28.2% (37/131), 36.0% (208/578), or 36.4% (43/118) harbored ESBL, respectively, whereas 41% (211) of 515 *E. coli* isolates, 34.3% (72) of 210 *K. pneumoniae*, and 4.9% (5) of 102 *P. mirabilis* had ESBL. In general, the isolates from a nursing home or those with ESBL had lower antimicrobial susceptibility rates than those from the community or those without ESBL production. Only amikacin, piperacillin/tazobactam, ertapenem, and imipenem/meropenem were active against >90% *Enterobacteriaceae* isolates, irrespective of ESBL production.
Conclusion: ESBL production was common among clinical *Enterobacteriaceae* isolates, especially *E. coli* or those isolated from nursing home residents.

Introduction

Extended-spectrum beta-lactamases (ESBLs) are the enzymes produced by certain bacteria, especially among *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, and are able to hydrolyze extended-spectrum cephalosporins, such as ceftazidime, ceftriaxone, cefotaxime, and oxyimino-monobactams.[1] The prevalence rate of ESBL-producing *Enterobacteriaceae* has been increasing in recent years. In a review article, it is estimated that 5—8% of *E. coli* isolates from Korea, Japan, Malaysia, and Singapore had ESBL, while the figure was 12—24% in Taiwan, Thailand, the Philippines, and Indonesia.[1] In the Asia-Pacific region, an antimicrobial surveillance study, the Study for Monitoring Antimicrobial Resistance Trends (SMART), in 2008, collected 2370 unique aerobic and facultative gram-negative bacilli associated with intra-abdominal infections (IAIs) from 11 countries. High rates of ESBL-producing *E. coli* and *K. pneumoniae* were observed in China (59.1% and 34.4%, respectively), India (61.2% and 46.8%, respectively), and Thailand (53.0% and 23.1%, respectively), particularly those causing hospital-associated intra-abdominal infections.[2] It also revealed the ESBL prevalence increased from 13% in 2002 to 28% in 2006 among *Enterobacteriaceae*.[3] Likewise, in a 7-year study at a tertiary hospital in Taiwan, a significant increase was evident for ESBL production among *E. coli* (from 4.8% to 10.0%) and *K. pneumoniae* (from 15.0% to 23.4%) isolates.[4]

Though ESBL-producing *E. coli* and *K. pneumoniae* were increasing, inappropriate antibiotic therapy was often noted and was associated with high mortality and prolonged hospitalization. In our previous study of 111 adults with bacteremic pneumonia caused by ESBL-producing *E. coli* and *K. pneumoniae*, 54.1% patients received inappropriate empiric antimicrobial therapy which was linked to a poor prognosis.[5] Moreover, inappropriate empirical antibiotics therapy has been often prescribed in cases of ESBL-producing *E. coli*, *K. pneumoniae*, or *P. mirabilis* bacteremia in the emergency department and was associated with longer hospital stay.[6] Selection of appropriate antimicrobials against *E. coli*, *K. pneumoniae*, and *P. mirabilis*, especially those with ESBL-production, therefore, was of clinical significance.

In this study we aim to study the antimicrobial susceptibility of clinical *E. coli*, *K. pneumoniae*, or *P. mirabilis* isolates in hospitalized patients with community-onset infections, in order to guide clinicians to select appropriate empirical antibiotics.

Methods

A retrospective study was performed at the Department of Health and Welfare, Tainan Hospital, a district hospital at southern Taiwan. Patients admitted between July 2009 and June 2011 were included if they were older than 18 years, admission via the emergent department, and blood, sputum, or urine culture that revealed one of either *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Clinical data, including age, sex, and sample category, were retrieved from the electronic database. The information of the patient's source was mandatorily queried and recorded for all visitors in the emergency department.

Microbiology analysis

Urine and sputum samples were transported to the laboratory and refrigerated at 4°C, and processed within 12 hours according to the following procedures. A calibrated loop was used and seeded in a 1-μL aliquot to 90-mm petri dishes containing CHROMagar (Becton Dickinson, Franklin Lakes, NJ, USA). Isolates were identified by the morphology and color in the chromogenic agar, followed by the confirmation of species identification by BD GNB ID or BD E/NF crystal kit (Becton Dickinson). Blood cultures were processed in the BD BACTEC 9240 system (Becton Dickinson). Antibiotic susceptibility was determined using the disk diffusion method in accordance with the procedures of the Clinical and Laboratory Standards Institute (CLSI), interpreted as being susceptible according to the susceptible zone criteria of CLSI issued in 2011 (M100-S21).[7] The drugs tested included ampicillin, ampicillin/sulbactam, gentamicin, amikacin, cefazolin, cefuroxime, ceftriaxone,

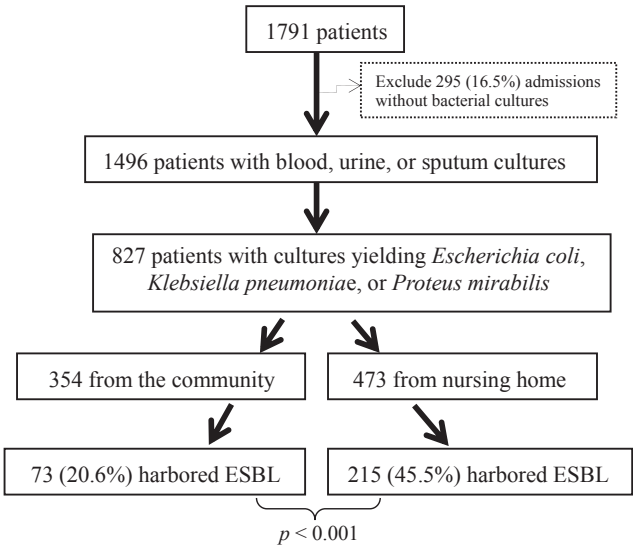

Figure 1. Community-acquired and nursing home-acquired extended spectrum beta-lactamase-producing *Enterobacteriaceae* isolates included in the present study. ESBL = extended spectrum beta-lactamase.

ceftazidime, cefepime, ciprofloxacin, ertapenem, imipenem, or meropenem. A difference of ≥5 mm between the zone diameters of either of cephalosporin disks and their respective cephalosporin/clavulanate disks was taken to be phenotypic confirmation of ESBL production.

Data analysis

Only one clinical isolate was included from each admission. From each patient only the first isolate from the infection site was included. If there was a blood isolate in addition to concurrent urine or sputum isolates, only the blood isolate was taken into account. If urine and sputum yielded the growth of different species, only one was included, according to the clinical diagnosis at admission.

Statistical analysis

Statistical analysis was performed by the statistical software (SPSS, version 13.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics, including the means, standard deviations, and ranges, were used to analyze the continuous variables. For categorical variables, the percentages and confidence intervals were used. Independent *t* test was used for the continuous variables and a Chi-square test or Fisher's exact test for the categorical variables. A two-tailed *p* value < 0.05 was considered to be statistically significant.

Results

During the study period, there were 1791 patients admitted to the study hospital, of which 295 (16.5%) had no pathogens isolated from blood, urine, or sputum (Figure 1). *E.*

Table 1 Clinical characters of 827 hospitalized adults infected by *Escherichia coli*, *Klebsiella pneumoniae*, or *Proteus mirabilis*.

Characters	Case number (%)			*p*
	Total, *n* = 827	Community, *n* = 354	Nursing home, *n* = 473	
Sex, male	337 (40.7)	120 (33.9)	217 (45.9)	0.001
Clinical specimen				0.009
Urine	578 (69.9)	235 (66.4)	343 (72.5)	
Sputum	118 (14.3)	47 (13.3)	71 (15.0)	
Blood	131 (15.8)	72 (20.3)	59 (12.5)	
Bacterial species				0.35
E. coli	515 (62.3)	227 (64.1)	288 (60.9)	
K. pneumoniae	210 (25.4)	90 (25.4)	120 (25.4)	
P. mirabilis	102 (12.3)	37 (10.5)	65 (13.7)	
Susceptibility rate				
Ampicillin/sulbactam	319 (38.6)	194 (54.8)	125 (26.4)	<0.001
Piperacillin	320 (38.7)	184 (52.0)	136 (28.8)	<0.001
Piperacillin/tazobactam	809 (97.8)	348 (98.3)	461 (97.5)	0.41
Cefazolin	253 (30.6)	161 (45.5)	92 (19.5)	<0.001
Cefuroxime	397 (48.1)	237 (66.9)	160 (33.8)	<0.001
Cefotaxime	497 (60.1)	265 (74.9)	232 (49.0)	<0.001
Ceftazidime	499 (60.3)	264 (74.6)	235 (49.7)	<0.001
Cefepime	534 (64.6)	280 (79.1)	254 (53.7)	<0.001
Gentamicin	401 (48.5)	233 (65.8)	168 (35.5)	<0.001
Amikacin	781 (94.4)	348 (98.3)	433 (91.5)	<0.001
Ciprofloxacin	371 (44.9)	218 (61.6)	153 (32.3)	<0.001
Ertapenem	814 (98.4)	350 (98.9)	464 (98.1)	0.38
Imipenem or meropenem	827 (100)	354 (100.0)	473 (100.0)	1.00
ESBL production	288 (34.8)	73 (20.6)	215 (45.5)	<0.001

ESBL = extended spectrum beta-lactamase.

coli, *K. pneumoniae*, or *P. mirabilis* was isolated from a total of 827 (55.3%) patents, which were included in the present study. Of 473 *Enterobacteriaceae* isolates from nursing homes, 215 (45.5%) exhibited the ESBL production phenotype, and in contrast only 73 (20.6%) of 354 isolates acquired in the community did ($p < 0.001$). Patient age of those from nursing homes was similar to those from the community (75.3 years vs. 73.9 years, $p = 0.23$; Table 1). Nevertheless female predominance was more evident in those from the community than nursing home residents (66.1% vs. 54.1%, $p = 0.001$).

Clinical isolates in the present study were obtained from urine (578 isolates, 69.9%), blood (131, 15.8%), and sputum (118, 14.3%), as shown in Table 2. Of urine and blood isolates, *E. coli* predominated, accounting for 66.6% and 71.0%, respectively. However, *K. pneumoniae* (55.1%) was the major pathogen of sputum isolates.

Of clinical specimens with *Enterobacteriaceae*, more urine specimens (72.5% vs. 66.4%) but less blood samples (12.5% vs. 20.3%) were noted in nursing home residents than those in the community. Species distribution of the isolates from nursing home or the community was similar, with the predominance of *E. coli*. Nevertheless, susceptibility rates of bacterial isolates of the same species from nursing home to all drugs but piperacillin/tazobactam, ertapenem, or imipenem were significantly lower than those from the community (Table 1).

Among *E. coli* isolates acquired in the community, ciprofloxacin nonsusceptibility rates varied among those from different specimens, ranging from 22.0% in 93 blood

Table 2 Ciprofloxacin susceptibility and extended spectrum beta-lactamase production in different species of urine, sputum, or blood isolates.

Microbiological characteristics	Community, $n = 354$	Nursing home, $n = 473$	p
Urine isolates, $n = 578$			
Escherichia coli, $n = 385$ (66.6%)	163	222	
Ciprofloxacin-nonsusceptibility	80 (49.1)	169 (76.1)	<0.001
ESBL production	41 (25.2)	117 (52.7)	<0.001
Both[a]	40 (24.5)	111 (50.0)	<0.001
Klebsiella pneumoniae, $n = 114$ (19.7%)	41	73	
Ciprofloxacin-nonsusceptibility	18 (43.9)	21 (65.8)	0.02
ESBL production	11 (26.8)	34 (46.6)	0.04
Both[a]	10 (24.4)	32 (43.8)	0.04
Proteus mirabilis, $n = 79$ (13.7%)	31	48	
Ciprofloxacin-nonsusceptibility	5 (16.1)	14 (29.2)	0.19
ESBL production	2 (6.5)	3 (6.2)	0.97
Both[a]	1 (3.2)	3 (6.2)	0.55
Sputum isolates, $n = 118$			
Escherichia coli, $n = 37$ (31.3%)	14	23	
Ciprofloxacin-nonsusceptibility	11 (78.6)	19 (82.6)	0.76
ESBL production	6 (42.9)	16 (69.6)	0.11
Both[a]	6 (42.9)	14 (60.9)	0.29
Klebsiella pneumoniae, $n = 65$ (55.1%)	30	35	
Ciprofloxacin-nonsusceptibility	6 (20.0)	25 (71.4)	<0.001
ESBL production	5 (16.7)	16 (45.7)	0.01
Both[a]	4 (13.3)	15 (42.9)	0.01
Proteus mirabilis, $n = 16$ (13.6%)	3	13	
Ciprofloxacin-nonsusceptibility	0 0	3 (23.1)	0.36
ESBL production	0	0	
Both[a]	0	0	
Blood isolates, $n = 131$			
Escherichia coli, $n = 93$ (71.0%)	50	43	
Ciprofloxacin-nonsusceptibility	11 (22.0)	34 (79.1)	<0.001
ESBL production	5 (10.0)	26 (60.5)	<0.001
Both[a]	3 (6.0)	26 (60.5)	<0.001
Klebsiella pneumoniae, $n = 31$ (23.7%)	19	12	
Ciprofloxacin-nonsusceptibility	4 (21.1)	7 (58.3)	0.04
ESBL production	3 (15.8)	3 (25.0)	0.53
Both[a]	3 (15.8)	2 (16.7)	0.95
Proteus mirabilis, $n = 7$ (0.2%)	3	4	
Ciprofloxacin-nonsusceptibility	1 (33.3)	1 (25.0)	0.81
ESBL production	0	0	
Both[a]	0	0	

[a] Co-existing ESBL production and ciprofloxacin-nonsusceptibility phenotype.
ESBL = extended-spectrum beta-lactamase.

Table 3 Characters of hospitalized adults infected by *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates with or without extended spectrum beta-lactamase production.

Characteristics	ESBL, $n = 288$	No ESBL, $n = 539$	p
Age (y)	75.7 ± 13.4	74.2 ± 15.9	0.16
Sex, male	149 (51.7)	188 (34.9)	<0.001
Clinical specimen			0.23
Urine	208 (72.2)	370 (68.6)	
Sputum	43 (14.9)	75 (13.9)	
Blood	37 (12.8)	94 (17.4)	
Bacterial species			<0.001
E. coli	211 (73.3)	304 (56.4)	
K. pneumoniae	72 (25.0)	138 (25.6)	
P. mirabilis	5 (1.7)	97 (18.0)	
Antimicrobial susceptibility			
Amikacin	262 (91.0)	519 (96.3)	0.001
Gentamicin	48 (16.7)	353 (65.5)	<0.001
Ampicillin/sulbactam	4 (1.4)	315 (58.4)	<0.001
Piperacillin/tazobactam	271 (94.1)	538 (99.8)	<0.001
Ertapenem	280 (97.2)	534 (99.1)	0.04
Imipenem or meropenem	211 (100.0)	72 (100.0)	1.00
Ciprofloxacin	18 (6.2)	353 (65.5)	<0.01

Data are presented as *n* (%) or mean ± standard deviation.
ESBL = extended spectrum beta-lactamase.

isolates, 49.1% in 385 urine isolates, to 78.6% in 37 sputum isolates (Table 2). By contrast, ciprofloxacin nonsusceptibility rates of community-acquired *K. pneumoniae* isolates ranged from 20.0% in 65 sputum isolates, 21.1% in 31 blood isolates, to 43.9% in 114 urine isolates. Of 37 community-acquired *P. mirabilis* isolates, mainly urine isolates, 16.2% (31) were not susceptible to ciprofloxacin.

In total, 288 (34.8%) isolates exhibited ESBL production phenotype. By species, 41.0% (211) of 515 *E. coli* isolates, 34.3% (72) of 210 *K. pneumoniae*, and 4.9% (5) of 102 *P. mirabilis* were ESBL producers. By clinical specimen, 36.0% (208) of 578 urine isolates, 36.4% (43) of 118 sputum isolates, and 28.2% (37) of 131 blood isolates were ESBL producers. The isolates from nursing homes were more likely to have ESBL production (45.5%, 215/473 vs. 20.6%, 73/354; $p < 0.001$; Table 1) or coexisting ESBL production and ciprofloxacin nonsusceptibility phenotype (42.9%, 203/473 vs. 18.9%, 67/354; $p < 0.001$) than those from the community. Such a difference was evident for *E. coli* and *K. pneumoniae* isolates, but not *P. mirabilis* (Table 2).

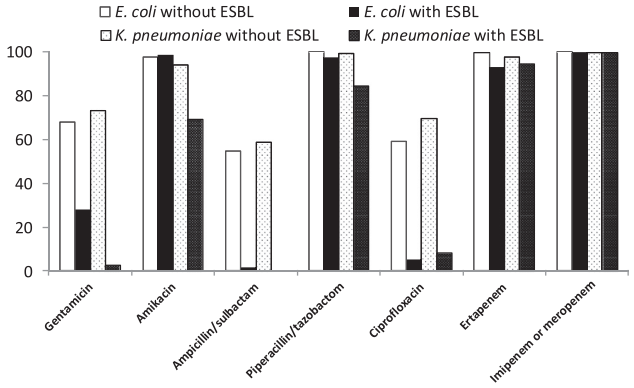

Figure 2. Antimicrobial susceptibility of *Escherichia coli* or *Klebsiella pneumoniae* isolates with and without extended spectrum beta-lactamase to seven drugs. ESBL = extended spectrum beta-lactamase

As compared with non-ESBL producers, ESBL producers usually had lower antimicrobial susceptibility rates, as shown in Table 3. Of note, only 1.4%, 6.2%, and 16.7% of ESBL producers were susceptible to ampicillin/sulbactam, ciprofloxacin, and gentamicin, respectively. Amikacin, piperacillin/tazobactam, ertapenem, and imipenem or meropenem were *in vitro* active against >90% of *Enterobacteriaceae* isolate, irrespective of ESBL production (Table 3). However, amikacin and piperacillin/tazobactam were *in vitro* active against 69.4% and 84.7%, respectively, of ESBL-producing *K. pneumoniae* isolates (Figure 2).

Discussion

The ESBL-production rate among *E. coli*, *K. pneumoniae*, and *P. mirabilis* isolates in our study was 20.6%, which was compatible to the ESBL prevalence rate of 12—24% among *E. coli* isolates in Taiwan and other Southeast Asia area.[1] In the SMART study, 2009—2010, ESBL-producing pathogens comprised 28.2% of all *Enterobacteriaceae* isolates.[8] Of note, one fifth of the isolates presumably acquired in the community had ESBL and such a finding warrants more detailed clinical studies. The substantial prevalence rate of ESBL production among *Enterobacteriaceae* isolates warrants clinical and public health attention.

Residence in a nursing home, as well as living in the hospitals, was associated with a higher risk for acquiring ESBL-producing *Enterobacteriaceae* isolates. Nursing home

residents often had nasogastric tube or urinary catheter placement and antimicrobial exposure. Among 393 patients with community-onset urinary tract infections due to *Enterobacteriaceae*, nasogastric tube placement and recent hospitalization were independently associated with the acquisition of ESBL-producing pathogens.[9] Similarly risk factors associated with ESBL production among community-onset *E. coli* bacteremia included recent antibiotic exposure and urinary catheter placement.[10] More specifically, previous exposure to oxyimino-cephalosporins was an independent predictor for ESBL-producer infection among *E. coli* bacteremia.[11]

The prevalence of ESBL phenotype varies not only among species, but also among the isolates from different body sites. Among clinical *Enterobacteriaceae* isolates in Taiwanese intensive care units in 2008, the prevalence of ESBL-producing isolates was 26% in *K. pneumoniae*, 16% *Serratia marcescens*, 14% *E. coli*, and 13% *P. mirabilis* isolates.[12] By contrast, these figures in our study were higher, 34% in *K. pneumoniae* and 41% *E. coli* isolates, suggestive of substantial antimicrobial resistance in the community. The majority of *Enterobacteriaceae* isolates with ESBL were *E. coli* isolates in the present study, in accordance with the results of the SMART study that of nearly 700 *Enterobacteriaceae* isolates with ESBLs, AmpC β-lactamases, or carbapenemases, *E. coli* predominated (63%),[13] reflecting its major role in human infection, frequent confronting of antimicrobial selective pressure, or both.

Most of our ESBL-producing *Enterobacteriaceae* isolates were nonsusceptible to ciprofloxacin, 93.8%, a figure higher than that in *Enterobacteriaceae* isolates without ESBL, 34.5%. Such a finding was in agreement with published data.[14,15] Coexisting fluoroquinolones resistance in our ESBL-producers may be related to previous frequent exposure to antimicrobial agents in nursing home residents. Although carbapenem resistance has been increasingly recognized in Taiwan,[16,17] there were few carbapenem-resistant *Enterobacteriaceae* isolates in this study. The carbapenem class remains a suitable choice for infectious diseases due to ESBL-producing *Enterobacteriaceae*. Besides, two drugs, amikacin and piperacillin/tazobactam, can be potential alternatives, if patients were allergic or intolerant to the carbapenems. Since there were significant differences in antibiotic susceptibility between clinical isolates from the community and nursing home, the clinicians in the emergency room setting should pay attention to the patients from nursing homes in empirical prescription of antimicrobial therapy. The antibiotics with a low susceptibility rate (<80%), including ciprofloxacin, may be avoided as initial therapy for nursing home residents with severe infections.

There were some limitations in our study. Firstly, it is a descriptive analysis of susceptibility data of clinical isolates and no clinical details, such as prior antimicrobial exposure or hospitalization, underlying disease, or clinical significance of each isolate, were available. However, our results could reflect resistance burden in community-onset infections due to *Enterobacteriaceae*. Secondly, only three species with confirmatory ESBL tests recommended by the CLSI were included for the study. Such results certainly underestimated the extent of ESBL production among all *Enterobacteriaceae* isolates. Thirdly, the CLSI susceptibility criteria changed years, so the susceptibility data may not be applicable or comparable to susceptible data form other geographic regions or study periods. Nevertheless, these regional susceptibility data of ESBL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis* isolates can guide the appropriate choice of empirical antibiotics.

In conclusion, ESBL production is not uncommon among frequently encountered community-onset *Enterobacteriaceae* isolates, especially *E. coli* and those isolated from nursing home residents. Concurrent ciprofloxacin resistance was common among ESBL-producers. To optimize the appropriateness of empirical antimicrobial therapy, continuing resistance surveillance in different clinical settings or hospitals is warranted.

Conflicts of interest

All authors declare no conflicts of interest.

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